

Ion Chromatography (IC) ICP-MS for Chromium Speciation in Natural Samples

Application

Environmental

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Abstract

Trace measurements of the element chromium (Cr) are of interest in a wide range of applications and matrices. In the environment, Cr exists in two different oxidation states, the trivalent Cr(III) cation and hexavalent Cr(VI) anion. In mammals, Cr(III) is an essential element involved in the regulation of glucose; however, the element in its hexavalent form demonstrates mutagenic and carcinogenic effects at relatively low levels. Because of this duality, total Cr measurements do not provide sufficient information to establish potential toxicity. In order to assess the potential toxicity of the Cr level in a sample, it is the Cr(VI) concentration that must be measured, rather than the total Cr concentration. A new method was developed to couple Ion Chromatography to Octopole Reaction Cell ICP-MS (inductively coupled plasma mass

spectrometry), to give a simple and reliable method for the separation and measurement of Cr(III) and Cr(VI), and so provide an accurate indication of the toxicity of the Cr level in a sample. This method has the merit of being applicable to high matrix samples, such as hard drinking water, due to the optimization of the sample preparation method and the chromatography. Also, the ICP-MS method provides excellent signal to noise, as a result of the removal of potentially interfering background species in the reaction cell, allowing the accurate determination of toxicologically useful levels of Cr(VI), at concentrations below 0.1 µg/L.

Introduction

The measurement of chromium toxicity is a requirement across a wide range of sample types, including drinking water, foodstuffs, and clinical samples (the latter used primarily to assess occupational exposure). However, it is the hexavalent form of Cr - Cr(VI) that is the toxic form, while the trivalent form - Cr(III) is an essential element for human nutrition. Methods to establish the potential toxicity of Cr must therefore determine the concentration of Cr(VI), rather than simply total Cr.

Two common approaches are used to address the issue: First, if the total Cr level measured is below the toxic level for Cr(VI), then it is reasonable to state that the Cr level will not be toxic, even if all of the Cr is present as Cr(VI). However, this approach can lead to a large number of false positives if samples contain a high concentration of Cr(III), so a more accurate approach is to separate and measure the Cr(VI) itself or, ideally, separate and measure both forms of Cr, giving an indication of the level of total Cr AND the level of toxic



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Cr(VI), from a single analysis.

Separating and detecting individual forms or species of elements is usually a straightforward analytical challenge, but Cr is an unusual case in this respect. This is because the common forms of Cr in natural samples such as water are chromate (CrO_4^{2-}) for Cr(VI) and chromic ion (Cr^{3+}) for Cr(III). Chromate is an anion and the chromic ion is cationic, so a single ion exchange method will not work for both forms under the same conditions. A further problem is that Cr(III) is the most stable oxidation state in samples such as water, whereas Cr(VI) ions are strong oxidizing agents and are readily reduced to Cr(III) in the presence of acid or organic matter. Consequently great care must be taken during sample collection, storage and preparation, to ensure that the Cr species distribution present in the original sample is maintained up to the point of analysis.

Experimental

The method described in this application note used an optimized sample stabilization method, in which the samples were incubated at 40 °C with EDTA, which forms a complex with the Cr(III), allowing a single chromatographic method to be used to separate the Cr(III)EDTA complex and the Cr(VI). The reaction to form the Cr(III)EDTA complex is dependent on the incubation time and temperature, with complete conversion occurring after less than 1 hour at 60 °C or 3 hours at 40 °C. Complete conversion did not occur even after 7 hours incubation

at room temperature.

Note that a relatively high concentration of EDTA is required for this method to be applicable to natural water samples, since other ions, such as Ca and Mg, which are commonly present at 10's or 100's mg/L in hard drinking water for example, would compete with the Cr(III) to form EDTA complexes, leading to low and matrix dependent Cr(III) recovery.

The combination of the separation of the Cr species using ion chromatography (IC), together with analysis of the separated species using ICP-MS, offers an ideal analytical method, as it permits the individual Cr species to be separated using a simple, low cost IC configuration. ICP-MS detection also allows the separated Cr species to be measured at extremely low concentrations, providing accurate assessment of exposure levels, even for natural or background Cr concentrations.

ICP-MS has excellent sensitivity and so is a good detector for many trace elements. The introduction of collision/reaction cells (CRC's) for ICP-MS allows Cr to be measured even more accurately and with better sensitivity, using the main isotope at mass 52, with removal of the primary matrix-based interferences ArC and ClOH. The sample preparation method, column type and chromatographic conditions used for Cr speciation are shown in Table 1. Note that, in addition to the stabilization of the samples with Na EDTA, EDTA was also added to the mobile phase, to stabilize the Cr(III) complex during separation. In addition, it was found that the use of pH 7 was essential for species stabilization and

Table 1. Chromatographic Conditions for Cr Speciation

Cr column	Agilent part number G3268A, 30 mm × 4.6-mm id
Mobile phase	5 mM EDTA (2Na), pH 7 adjust by NaOH
Flow rate	1.2 mL/min
Column temperature	Ambient
Injection volume	50–500 µL
Sample preparation	
Reaction temperature	40 °C
Incubation time	3 h
EDTA concentration	5–15 mM pH 7 adjust by NaOH

optimum chromatographic separation.

The IC configuration used for the work presented in this note is illustrated in Figure 1. Note that the nonmetal IC pump (Metrohm 818 IC Pump was used to deliver the mobile phase, but the sample loop was filled and switched using the optional Integrated Sample Introduction System (ISIS) of the Agilent 7500ce ICP-MS. While this configuration maintains the high precision and relatively high pressure of the IC pump, it provides a much simpler and lower-cost alternative to a complete IC or HPLC system,

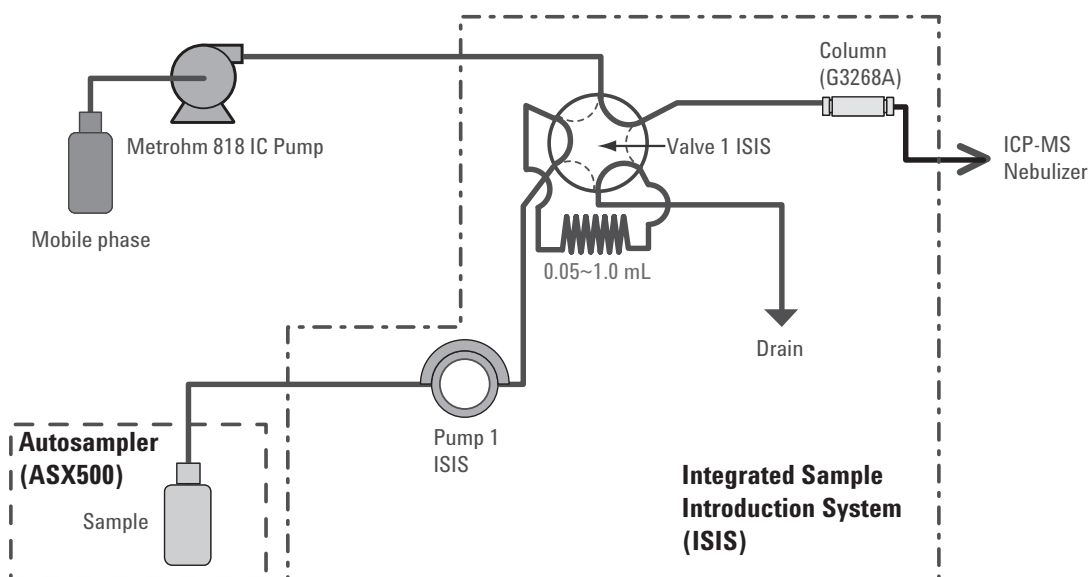


Figure 1. IC-ICP-MS configuration used for Cr speciation.

since only the IC pump module is required in addition to the ICP-MS system.

Results and Discussion

Under the conditions described above, with ICP-MS detection using the Agilent 7500ce in H₂ cell gas mode to remove the ArC and ClOH interferences on Cr at mass 52, detection limits (DLs) of <20 ng/L were obtained for the individual Cr species, as shown in Table 2. Many international regulations for hexavalent Cr specify a maximum allowable concentration of 1 µg/L, with a required DL of one-tenth of this level (100 ng/L), and even the small sample volume injection of 100 µL easily meets these

Table 2. DLs for Cr Species by IC-ICP-MS

Inject/ μ L	RT/min		Peak height/counts		Peak area/counts		DL (ng/L)*	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
50	0.79	2.09	8548	4261	1082295	914804	69.5	139.4
100	0.79	2.09	13688	7173	1704312	1525147	43.4	82.8
250	0.85	2.21	33967	20830	4939876	4546219	17.5	28.5
500	0.97	2.39	44870	37502	10268086	9398651	13.2	15.8

*Detection limits calculated as three times the peak-to-peak signal-to-noise as measured on standard chromatograms.

requirements. However, increasing the injection volume to 500 μ L allowed the DLs to be reduced to 13.2 ng/L for Cr(III) and 15.8 ng/L for Cr(VI).

For a simple standard solution, these conditions give an excellent signal to noise for both Cr species, as illustrated in Figure 2. This chromatogram shows the separation of the two Cr species each at a concentration of 0.1 μ g/L (ppb), using an injection

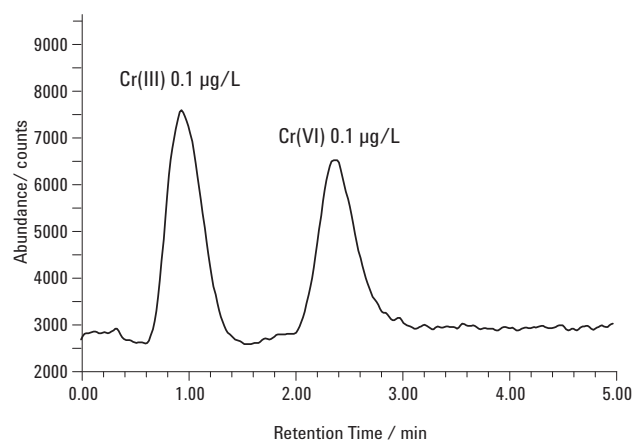


Figure 2. Separation and detection of Cr(III) and Cr(VI) at a concentration of 0.1 μ g/L each species.

volume of 500 μ L. Clearly the peaks are easily detected above the background and the baseline separation of the two species in a total time of about 3 minutes is also illustrated.

Using a series of synthetic standard solutions at low concentrations, a calibration was created for each of the two Cr species. Quantification was based on

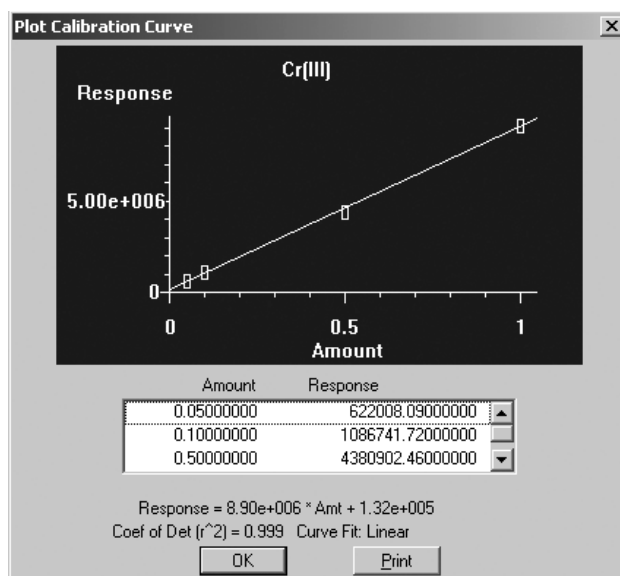


Figure 3. Calibration for Cr(III) - Standard concentrations 0.05, 0.1, 0.5 and 1.0 μ g/L.

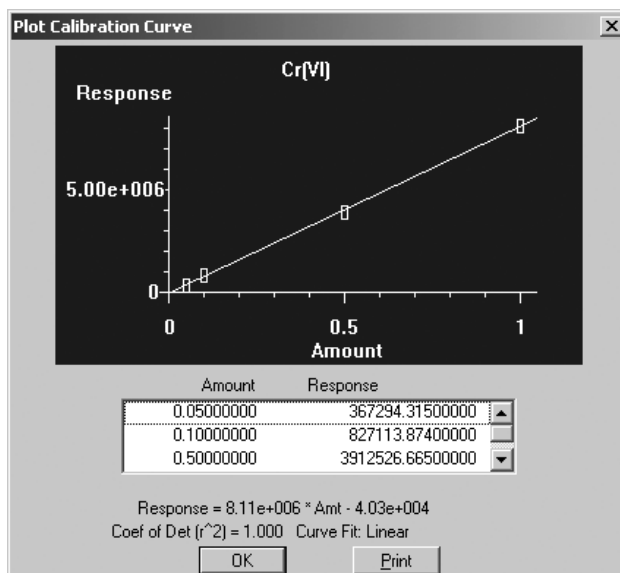


Figure 4. Calibration for Cr(VI) - Standard concentrations 0.05, 0.1, 0.5 and 1.0 μ g/L.

peak area. The calibrations obtained for Cr(III) and Cr(VI) are illustrated in Figures 3 and 4, respectively, each showing excellent sensitivity and linearity.

In addition to sensitivity, species stability, chromatographic separation and calibration linearity, for the method to be suitable for routine analysis, it is essential that it provides acceptable long-term stability. In chromatographic analysis, stability is governed by two factors, RT stability and peak area stability. The data in Table 3 illustrates both of these parameters and indicates that the stability of the method is certainly acceptable for routine operation.

Routine Analysis

Of more interest for the routine analysis of chromium species (or specifically hexavalent Cr) in natural water samples is the maintenance of this excellent sensitivity, stability and chromatographic separation in samples that contain a high concentration of other ions. In order to test the suitability of the method for these real-world sample types, the method was applied to the determination of both Cr species in both spiked and unspiked mineral water samples.

The first sample evaluated was a leading French mineral water referred to in this study as mineral water A. Figure 5 shows the chromatogram of the two Cr species in the unspiked and spiked samples of mineral water A. The major element composition of the water is also shown in the table inset in Figure 5, indicating the typical drinking water com-

Table 3. Stability of RT and Peak Area for Multiple 500 μ L Injections of 0.5 μ g/L Each Cr Species

Number	RT/min		Peak height/counts		Peak area/counts	
	Cr(III)-EDTA	Cr(VI)	Cr(III)-EDTA	Cr(VI)	Cr(III)-EDTA	Cr(VI)
1	0.969	2.338	23514	18437	5331427	4621752
2	0.969	2.338	22642	18784	5280683	4758462
3	0.969	2.338	22832	18615	5220349	4742259
4	0.952	2.338	24104	19944	5470760	4800723
5	0.969	2.372	22797	19203	5287094	4726640
6	0.969	2.405	23830	19328	5498172	4760285
7	0.985	2.338	23971	19479	5481984	4824934
8	0.969	2.338	23393	19675	5474510	4883193
9	0.969	2.355	23070	20097	5355106	4892160
10	0.969	2.372	23826	19896	5428247	4886400
Avg	0.97	2.38	23398	19346	5382833	4789681
STD	0.008	0.014	534.45	581.88	100413.18	85782.42
RSD%	0.80	0.57	2.28	3.01	1.87	1.79

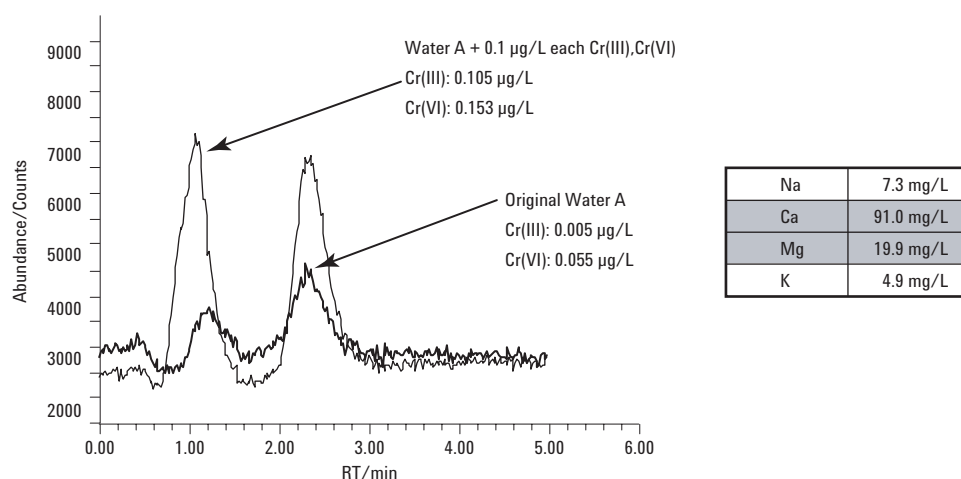


Figure 5. Major element composition (mg/L) and chromatogram for spiked and unspiked mineral water A.

position of about 100 mg/L Ca and between 5 mg/L and 20 mg/L of the other major elements K, Mg and Na.

The spike recovery data for mineral water A is shown in Table 4, indicating the very low level at which the Cr species were quantified (0.005 µg/L

Table 4. Spike Recovery Data for 0.1 µg/L Spikes of Cr(III) and Cr(VI) in Mineral Water A

Element	Original mineral water A	Found (µg/L)		Recovery (%)
		Spike added	Spike found	
Cr(III)	0.005	0.10	0.105	100.0
Cr(VI)	0.055	0.10	0.150	95.0

and 0.055 µg/L for Cr(III) and Cr(VI), respectively), and the excellent spike recovery accuracy for the low concentration spikes in this sample - better than 5% error in both cases.

The second mineral water sample analyzed was another French mineral water, referred to as mineral water B, which has among the highest levels of calcium and sulfates of any commonly available mineral water (over 450 mg/L Ca and more than 1000 mg/L sulfates). As for the mineral water A sample, mineral water B was analyzed with and

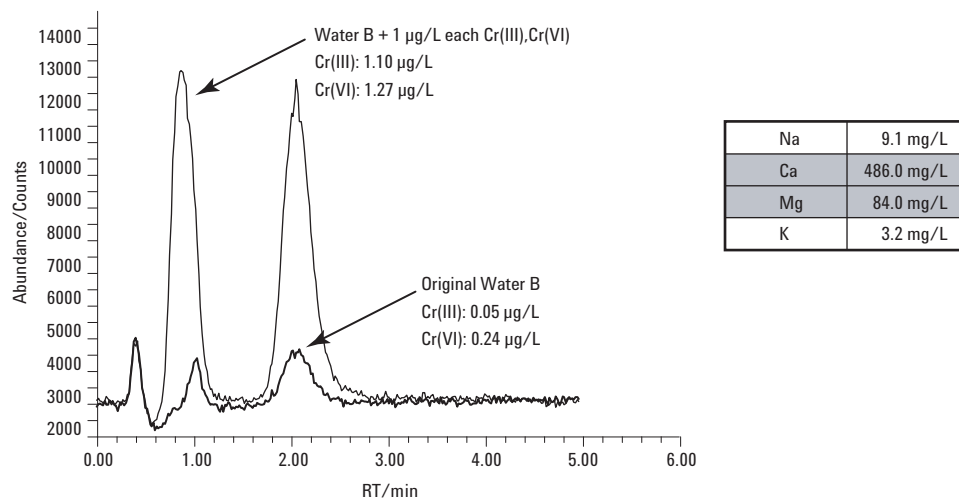


Figure 6. Major element composition (mg/L) and chromatogram for spiked and unspiked mineral water B.

without a spike of the two Cr species and the spike recovery was assessed. The results for the measured chromatograms are shown in Figure 6, while the spike recovery data are shown in Table 5.

As shown for the mineral sample A, the major element composition of the mineral water is shown as an inset in the chromatogram, illustrating the very high mineral levels in mineral water B. Despite these high major element levels, the optimized sample preparation and chromatographic method gave good chromatographic separation and excellent spike recovery results for both Cr species. A higher spike level was used for mineral water B, due to the higher baseline (unspiked) concentration for the Cr species in this sample.

The ability to recover low concentration spikes for both Cr species in such a high matrix sample indicates the effectiveness of the optimized method for sample stabilization, which ensures that a high enough concentration of EDTA is available for complete complexation of the Cr(III) species, even in the presence of a high level of competing ions.

Furthermore the accurate recovery of low concentration spikes of both species indicates that potential problems of species interconversion (reduction of Cr(VI) to Cr(III)) was avoided through the selection of an appropriate pH for the samples and the

mobile phase, together with the use of EDTA in the mobile phase as well as for sample stabilization. See Table 5.

Conclusions

A new method for the stabilization and analysis of Cr(III) and Cr(IV) in natural, high matrix water samples was developed and optimized with DLs in the region of 0.05 µg/L for 100-µL injections, or 0.015 µg/L for larger, 500-µL injections.

Reliable and stable separation of the Cr(III) and Cr(VI) species was achieved in a method taking approximately 3 minutes per sample and the separation and accurate quantification of the two species could be maintained even in the presence of a high concentration of competing ions, such as >500 mg/L mineral elements in the highly mineralized water.

Accurate and interference-free determination of Cr at the low concentrations (0.1 µg/L) required by international regulations was made possible by the simple and consistent operation of the Agilent 7500ce in reaction mode, using H₂ as a cell gas. This mode of operation does not preclude the simultaneous analysis of other analytes of interest, such as As, in contrast to the use of highly reactive cell gases such as CH₄ or NH₃.

Table 5. Spike Recovery Data for 1.0 µg/L Spikes of Cr(III) and Cr(VI) in a Highly Mineralized Water (B)

Element	Original mineral water B	Found (µg/L)		
		Spike added	Spike found	Recovery (%)
Cr(III)	0.05	1.0	1.10	105
Cr(VI)	0.24	1.0	1.27	102

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